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Original article

Cytotoxic potential of novel 6,7-dimethoxyquinazolines

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ABSTRACT

Herein, we report the synthesis and cytotoxicity of a series of substituted 6,7-dimethoxyquinazoline derivatives. The cytotoxic activity of all synthesized compounds has been evaluated against HCT116p53$^{+/+}$ and HCT116p53$^{-/-}$ colon cancer cells and a HEY ovarian cancer cell line naturally resistant to cisplatin. Nine of the tested compounds showed significant cytotoxicity in all cell lines at 10 μM. The most promising derivative (**7c**) showed IC_{50}values of 0.7 and 1.7 μM in the two colon cancer cell lines.

1. Introduction

2-Phenyl-4-quinolones (**PQ**) [1–4] and several flavonoids (**FL**) [5] are known to possess potent antimitotic activity (Fig. 1). These two classes of compounds, sharing a similar overall skeleton and differing in the nature of the heteroatoms, seem to act with the same mechanism based on kinase inhibition or the prevention of tubulin polymerization. The effects observed were similar to those recorded for antimitotic natural products such as colchicine, podophyllotoxin, and combretastatin 4-A. Thus due to their simple structural features, these compounds represent interesting candidates to be developed as antitumor agents. Lee and co-workers reported the synthesis and biological activity of variously substituted 2-arylquinazolinones (**AQ**) [6], which were considered as bioisosteres of **PQ** and **FL**. **AQ** were found to possess significant growth inhibitory action against a panel of colon cancer cell lines [7–9]. It was established that the contemporary presence of an aromatic substituent on position-2 of the quinazoline nucleus and 6,7-dimethoxy substitution are necessary for activity [7,10]. Amongst all the tested derivatives, compound **AQ_1** showed the highest cytotoxic activity with an $ED_{50} = 1.6$ μM against a human epidermoid carcinoma cell line KB-VIN [6]. This inspired us to use compound **AQ_1** as a lead molecule for the development of more potent anticancer agents with desirable pharmacokinetic properties.

In continuation of our studies aimed at the identification of small molecules with cytotoxic properties, we planned the synthesis of several quinazolin-4-one derivatives, maintaining the 6,7-dimethoxy substituents. In particular, 2-aryl, -benzyl, -arylamino and arylaminomethyl derivatives were prepared. Replacement of the oxygen carbonyl moiety of quinazolinone system by an amino or azido group was also explored. Several derivatives bearing a lipophilic *n*-butyl group at position-3 of the quinazoline system were also prepared.

2. Results and discussion

2.1. Chemical

Firstly 2-aryl-6,7-dimethoxy-4(3*H*)-quinazolinones (**4**) have been synthesized as reported in Scheme 1. Substituted benzoic acids (**1**) were condensed with 2-amino-4,5-dimethoxy benzamide (**2**) in presence of EDC or thionyl chloride to obtain the diamides (**3**). The diamides were subjected to cyclization under different experimental conditions to afford the desired 2-aryl-6,7-dimethoxyquinazolin-4(3*H*)-ones (**4**).

A similar strategy was utilized for the synthesis of compounds (**7**), in which the aryl group was linked to the quinazolinone system

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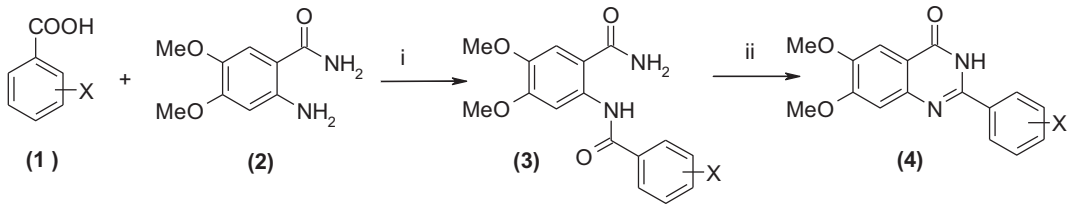


X = a) 3-NHCOMe; **b)** 2-Cl; **c)** 3-Cl; **d)** 3-OMe; **e)** 4-OMe; **f)** 3-Me; **g)** 4-Me

Scheme 1. Reagents and conditions: i) EDC, TEA, $CHCl_3$ or $SOCl_2$, 1,4 Dioxane; ii) Fuse or NaOH, CH_3OH, or *t*-BuONa, ethylene glycol, MW.

by a methylene group. Substituted phenylacetic acids (**5**) were condensed with the amide (**2**) to obtain the diamides (**6**), which gave the desired quinazolines (**7**) under basic conditions and microwave irradiation (Scheme 2).

A second series of compounds in which the quinazolinone and the aryl moieties were linked by aminomethylene moiety were obtained as depicted in Scheme 3. The methyl anthranilate derivative (**8**) was reacted with chloroacetonitrile in presence of dry hydrogen chloride to afford the 2-chloromethyl derivative (**9**), which gave the desired products (**11**) after reaction with the appropriate substituted anilines (**10**).

An *n*-butyl group was then introduced into position-3 of the quinazolinone moiety in order to improve lipophilicity of compounds (**4**) to give derivatives (**16**), according to Scheme 4. The benzoic acid derivatives (**12**) were transformed to acid chlorides by treatment with thionyl chloride. The acid chlorides were then treated with *n*-butylamine to give the desired amides (**13**). The nitro group in compounds (**13**) was reduced with iron powder to give the desired amines (**14**). The acid chlorides obtained from the benzoic acid derivatives (**1**) were reacted with the amino derivatives (**14**) to provide the diamides (**15**), which were cyclized under basic conditions to the desired products (**16**) (Scheme 4). The nitriles were converted into the tetrazoles (**15f** and **15g** from **15d** and **15e**, respectively), which were cyclized to the desired products (**16f** and **16g**).

It was also planned to replace the oxygen of the 4-keto group of quinazolinone ring with a basic amino group. Accordingly, the 4-keto derivatives (**4**) were treated with phosphorous oxychloride to give the corresponding 4-chloro derivatives (**17**), which in turn were converted into the azide derivatives (**18**) by treatment with sodium azide. Finally, the azide was reduced into corresponding amines (**19**) by catalytic hydrogenation (Scheme 5).

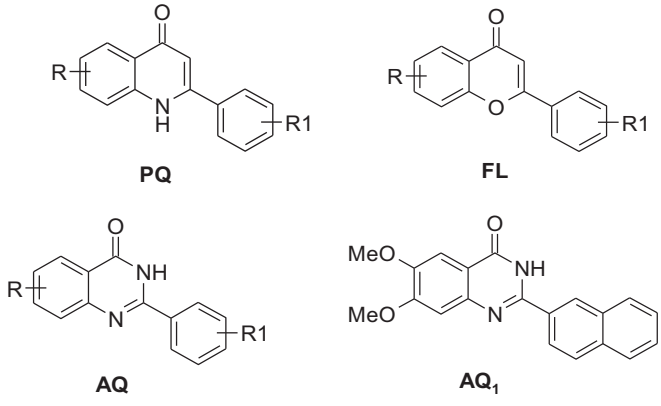


Fig. 1. General structures of reference compounds 2-phenyl-4-quinolones **PQ**, flavonoids **FL**, 2-arylquinazolinones **AQ**, and the structure of the lead compound $\mathbf{AQ_1}$.

Replacement of the methylene bridge with a basic amino function was also taken into consideration. The 4-amino-2-chloroquinazoline (**21**) [11] was reacted with substituted anilines (**20**) to give compounds (**22**), as reported in Scheme 6.

Physical data of the final synthesized final compounds is summarized in Table 1.

2.2. Cytotoxicity

Twenty-nine compounds selected on the basis of their structural features were initially tested in three human cancer cell lines from different tumor origins, two colon cancer cells HCT116p53$^{+/+}$ and HCT116p53$^{-/-}$, and an ovarian cancer cell line HEY, naturally resistant to cisplatin. Nine compounds (**7a**–**7c**, **18b**, **18c**, **18e**–**18g** and **22b**) showed >50% cytotoxicity at 10 μM (Table 2) in one or more of the cell lines. These compounds were tested at multiple doses to determine their corresponding IC_{50} values (Table 3).

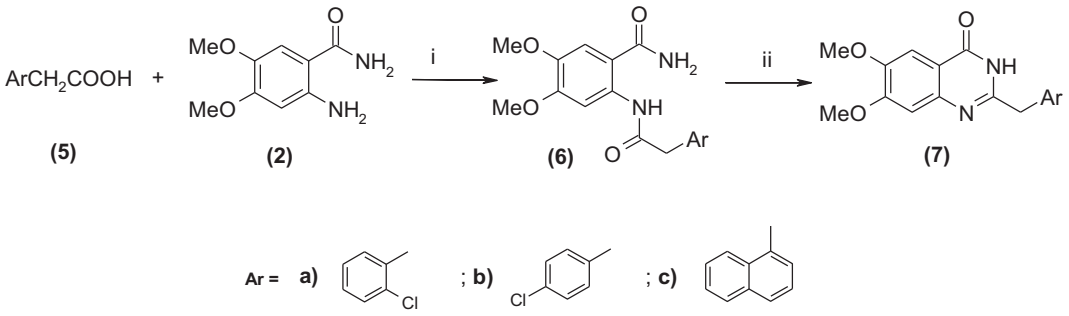
Compound **7c** was found to be the most potent derivative against HCT116p53$^{+/+}$ and HCT116p53$^{-/-}$ cell lines with IC_{50} values of 1.7 and 0.7 μM, respectively.

Compound **22b** was the second most potent derivative against the same cell lines with IC_{50} values of 6 and 5 μM, respectively. Compounds **18b**, **18c**, **18e**, **18f**, **18g**, **7a** and **7b** showed only moderate activities against HCT116 p53$^{+/+}$ cells while compound **18c**, **18e** and **18g** showed moderate activity against HCT116 p53$^{-/-}$ cells.

Considering their cytotoxicity profile, we have examined the effect of compounds **7c** and **22b** on tumor cell viability, using colony formation assay. The exposure of the two colon cancer cell lines to micromolar concentrations of these compounds resulted in a significant inhibition of colony formation. Compounds **7c** and **22b** completely abolished cell growth, at doses above 1 and 5 μM, respectively (Fig. 2).

Cell cycle perturbation induced by compound **22b** was examined in HCT116 p53$^{+/+}$ and HCT116 p53$^{-/-}$ colon cancer cells. The analysis of DNA profile by flow cytometry indicated that compound **22b** induced partial cell cycle arrest in G2/M phase in colon cancer cell lines. Treatment with compound **22b** caused retention of 34.5% of cells in G2/M phase in the HCT116 p53$^{-/-}$ cell, after 48 h treatment compared to 24.5 in the controls. The property of these compounds to induce cell cycle arrest makes them promising agents for combination with drugs acting at different stages of cell cycle (Fig. 3).

A few considerations can be drawn regarding the structure–activity relationships for the newly synthesized class of compounds. It seems that the presence of the oxygen or the amino group at position 4 does not significantly impair the cell growth inhibitory activity. Compounds bearing a one-atom linker (**7a**–**7c**, **22b**) between the quinazolinone system and the aryl ring showed higher activity. The *n*-butyl lipophilic moiety, at position-3 of the quinazoline ring, adversely affects the cell inhibiting activity. The



Scheme 2. Reagents and conditions: i) $SOCl_2$, 1,4 Dioxane; ii) *t*-BuONa, ethylene glycol, MW.

best structural combination required for activity seems to be the presence of an aryl group, substituted or not, appended to the quinazoline system by a methylene or an NH group.

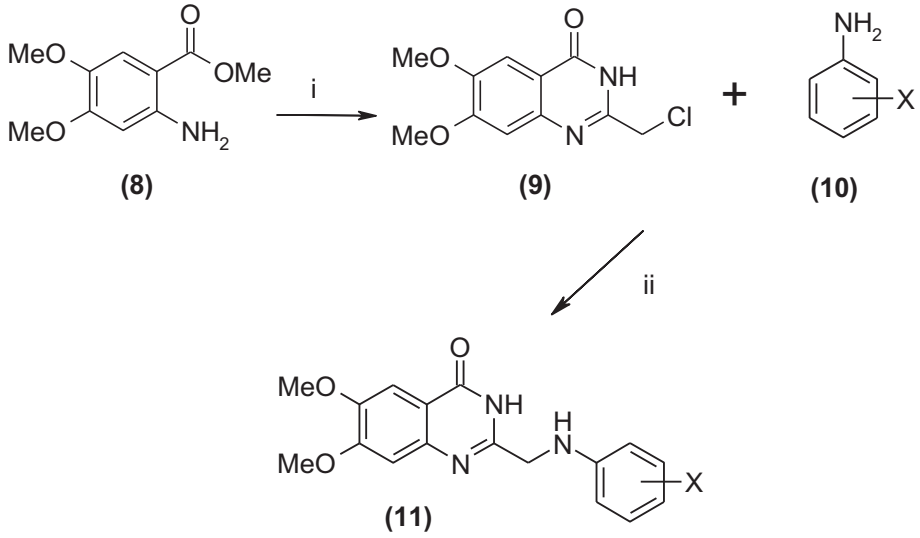
3. Conclusion

In this study, we designed and synthesized a series of substituted 6,7-dimethoxyquinazoline derivatives. The newly synthesized compounds have been tested against three human cancer cell lines for initial cytotoxicity evaluation. Structure–activity relationship study revealed that the substitution of the hydrogen at position-3 with a lipophilic *n*-butyl group reduces the activity, whereas the introduction of a bulky aromatic group as substituent at position-2 of the quinazoline ring, is favorable for cell inhibitory activity. In particular, the two compounds **7c** and **22b** showed an interesting cytotoxic profile. Their biological activities were further assessed by colony formation assay to confirm cytotoxicity. Flow cytometry analysis demonstrated that compound **22b** exerted cell cycle arrest in HCT116 colon cancer cells. Considering their cytotoxicity profiles, these compounds seem to have potentials as novel chemotherapeutics and, therefore, warrant further preclinical and mechanistic investigation.

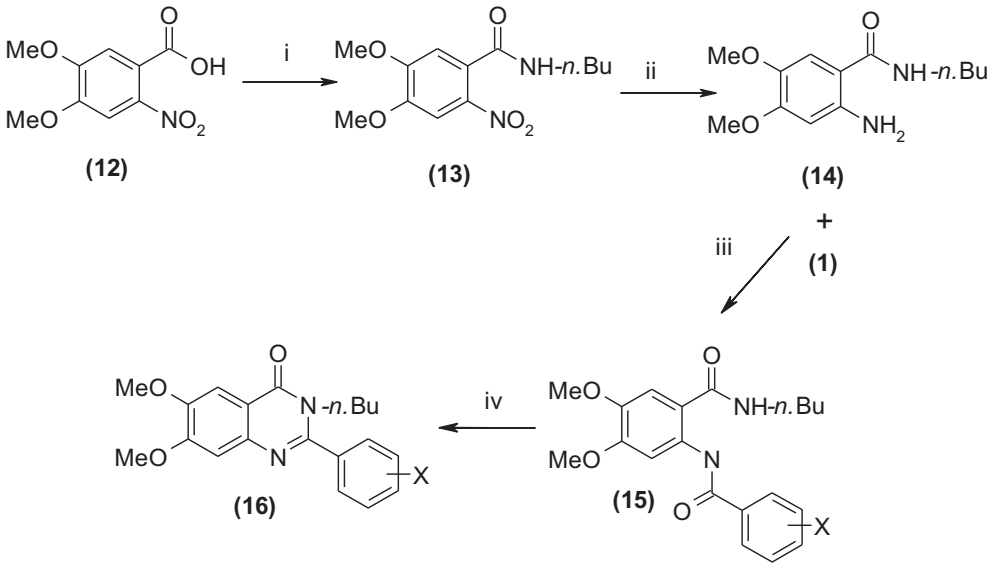
4. Experimental

4.1. Chemistry

Melting points were determined using a Veego make silicon oil bath-type melting point apparatus and are uncorrected. Purity of the compounds and completion of reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck), visualizing with ultraviolet light or iodine vapors. The IR spectra were recorded using KBr disc method on a Shimadzu FT-IR, Model 8300. The ^{1}H NMR spectra (on a Bruker 300/400 MHz spectrometer) were recorded in DMSO. Chemical shifts were reported as part per million (δ ppm) using tetramethylsilane (TMS) as an internal standard. The assignment of exchangeable protons was confirmed by the D_2O exchange studies wherever required. Mass spectral data was obtained on a QTRAP Applied Biosystem SCIEX spectrometer or on JEOL-SX-102 instrument (JEOL, Tokyo, Japan) by fast atom bombardment (FAB positive mode). Elemental analyses were obtained on Carlo Erba, Italy and Perkin–Elmer instruments.



Scheme 3. Reagents and conditions: i) $ClCH_2CN$, Dry HCl gas, 1,4 Dioxane; ii) K_2CO_3, DMF.



X = a) 4-$NHSO_2Me$; b) 3-$NHSO_2Me$; c) 4-NHCOMe; d) 4-CN; e) 3-CN; f) 4-(1*H*-tetrazol-5-yl); g) 3-(1*H*-tetrazol-5-yl)

Scheme 4. Reagents and conditions: i) $SOCl_2$, *n*-$BuNH_2$, THF; ii) Fe, NH_4Cl, CH_3OH; iii) $SOCl_2$, TEA, 1,4 Dioxane; iv) NaOH, CH_3OH.

Spectral data (IR, NMR, and MS) confirmed the structures of the synthesized compounds and the purity were ascertained by microanalysis. Microwave reactions were performed in CEM-Discovery, USA microwave reactor.

*4.1.1. 2-(3-Acetamidobenzamido)-4,5-dimethoxybenzamide (**3a**)*

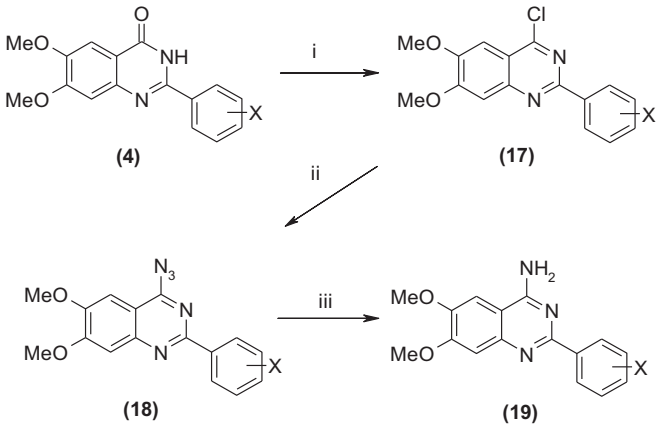
3-Acetamidobenzoic acid (**1a**) (0.46 g, 2.55 mmol) was dissolved in chloroform (50 mL) and the solution was cooled to 10–15 °C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (0.59 g, 3.06 mmol) was added in portions and the reaction mixture was stirred for 45 min at 10–15 °C. 2-Amino-4,5-dimethoxybenzamide (**2**) (0.5 g, 2.55 mmol) and triethylamine (TEA) (0.88 mL, 6.38 mmol) were subsequently added at 10–15 °C. The reaction mixture was stirred at rt for 15 h, washed with a solution of 5% hydrochloric acid (2 × 50 mL), aq sodium bicarbonate (5%, 2 × 50 mL) and water (2 × 50 mL), sequentially. The organic layer was dried, concentrated under reduced pressure and the crude solid so obtained was purified by recrystallization from chloroform–methanol to afford compound **3a** (0.65 g, 72%): mp 195–197 °C (dec.); IR (KBr): 3271, 1668, 1633, 1539, 1404, 1313, 1242, 1180 and 1008 cm^{-1}.

*4.1.2. 2-(2-Chlorobenzamido)-4,5-dimethoxybenzamide (**3b**)*

A mixture of 2-chlorobenzoic acid (**1b**) (0.61 g, 3.92 mmol) and thionyl chloride (1 mL) was heated for 2 h. The excess of thionyl chloride was recovered under reduced pressure and the residue so obtained was dissolved in dry dioxane (1 mL). The solution was then dropwise added to mixture of 2-amino-4,5-dimethoxybenzamide (**2**) (0.7 g, 3.57 mmol) and TEA (1.2 mL, 8.92 mmol) in dry dioxane (2 mL) at 10 °C. The reaction mixture was stirred at rt for another 1 h and quenched into cold water (50 mL). The precipitate formed was filtered, washed with water and dried to obtain compound **3b** (0.85 g, 71%): mp 213–215 °C (dec.); IR (KBr,): 3406, 3305, 1658, 1645, 1612, 1525, 1253, 1078 and 1039 cm^{-1}.

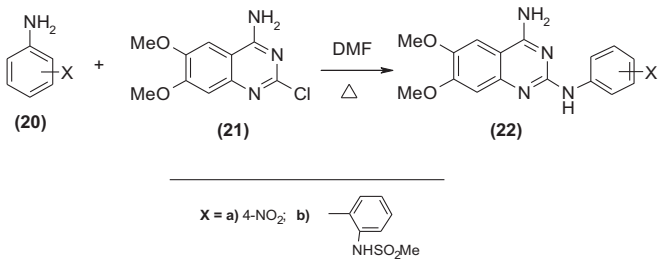
*4.1.3. 2-(3-Chlorobenzamido)-4,5-dimethoxybenzamide (**3c**)*

Following a procedure identical to the one as described for **3b**, but using 3-chlorobenzoic acid (**1c**) (0.60 g, 3.92 mmol), compound **3c** was obtained as a pale yellow solid (0.60 g, 50%): mp



X = **b)** 2-Cl; **c)** 3-Cl; **d)** 3-OMe; **e)** 4-OMe; **f)** 3-Me; **g)** 4-Me

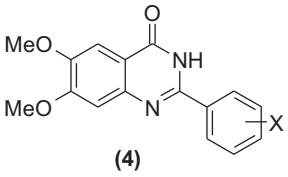
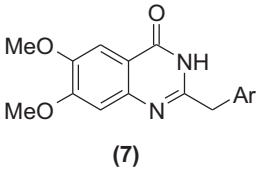
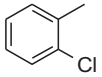
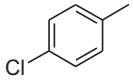
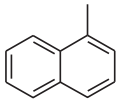
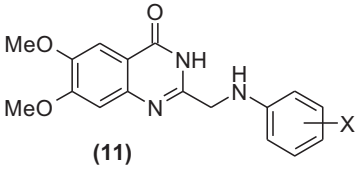
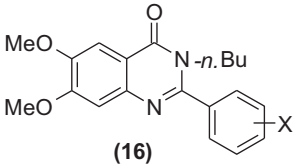
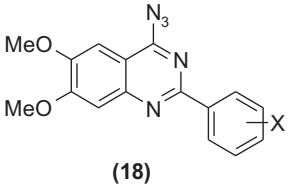
Scheme 5. Reagents and conditions: i) $POCl_3$, DMF; ii) NaN_3, DMF, mw; iii) H_2/Pd/(C), CH_3OH.



X = a) 4-NO_2; **b)** 3-$NHSO_2Me$

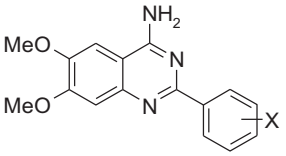
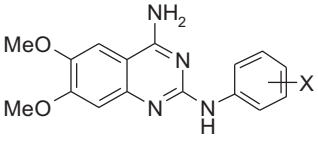
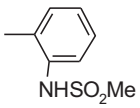
Scheme 6.

Table 1
Chemical structure and physicochemical data of final compounds.

Structures	No.	X	Mol. wt.	Mol. formula	Mp (°C)	IR (KBr)cm^{-1}
 (4)	**4a**	3-NHCOMe	339.35	$C_{18}H_{17}N_3O_4$	>310	3301, 1658, 1625, 1604, 1533, 1436, 1236, 1168 and 1010
	4b	2-Cl	316.75	$C_{16}H_{13}ClN_2O_3$	275–277 (dec.)	1654, 1614, 1488, 1438, 1388, 1282, 1215, 1176 and 1028
	4c	3-Cl	316.75	$C_{16}H_{13}ClN_2O_3$	>310	1652, 1614, 1596, 1490, 1390, 1276, 1178, 1103 and 1031
	4d	3-OMe	312.33	$C_{17}H_{16}N_2O_4$	262–264 (dec.) [12]	1651, 1616, 1494, 1436, 1390, 1284, 1249, 1053 and 1029
	4e	4-OMe	312.33	$C_{17}H_{16}N_2O_4$	255–258 (dec.)	1665, 1619, 1490, 1441, 1386, 1304, 1252, 1176 and 1030
	4f	3-Me	296.33	$C_{17}H_{16}N_2O_3$	278–280 (dec.)	1652, 1610, 1492, 1434, 1388, 1278, 1178 and 1037
	4g	4-Me	296.33	$C_{17}H_{16}N_2O_3$	285–287 (dec.)	1662, 1610, 1496, 1436, 1388, 1244, 1174 and 1033
 (7)	**7a**		330.77	$C_{17}H_{15}ClN_2O_3$	245–249 (dec.)	1668, 1612, 1488, 1286, 1236, 1218, 1099, 1166 and 1037
	7b		330.77	$C_{17}H_{15}ClN_2O_3$	243–245 (dec.)	1665, 1612, 1558, 1510, 1473, 1390, 1242, 1176 and 1034
	7c		346.39	$C_{21}H_{18}N_2O_3$	262–264 (dec.)	1666, 1612, 1434, 1390, 1274, 1242, 1218, 1078 and 1037
 (11)	**11a**	4-COOH	355.35	$C_{18}H_{17}N_3O_5$	229–231 (dec.)	3373, 1677, 1648, 1604, 1487, 1386, 1271, 1172 and 1010
	11b	3-NHSO$_2$Me	404.45	$C_{18}H_{20}N_4O_5S$	297–299	3222, 1666, 1612, 1595, 1498, 1358, 1230, 1149 and 1022
	11c	4-NHSO$_2$Me	404.45	$C_{18}H_{20}N_4O_5S$	267–269 (dec.)	3255, 1674, 1610, 1496, 1463, 1328, 1278, 1143 and 1089
	11d	4-OMe	341.37	$C_{18}H_{19}N_3O_4$	243–245 (dec.)	3354, 1654, 1612, 1508, 1483, 1386, 1251, 1095 and 1022
 (16)	**16a**	4-NHSO$_2$Me	431.51	$C_{21}H_{25}N_3O_5S$	184–189	3401, 2931, 1695, 1633, 1602, 1377, 1272, 1166 and 1025
	16b	3-NHSO$_2$Me	431.51	$C_{21}H_{25}N_3O_5S$	125–127	3419, 2933, 1697, 1639, 1602, 1357, 1272, 1178 and 1010
	16c	4-NHCOMe	395.46	$C_{22}H_{25}N_3O_4$	198–199	3282, 2931, 1668, 1645, 1600, 1533, 1265, 1178 and 1033
	16f	4-(1*H*-tetrazol-5-yl)	406.45	$C_{21}H_{22}N_6O_3$	220–223	2931, 1670, 1608, 1498, 1438, 1377, 1240, 1078 and 1022
	16g	3-(1*H*-tetrazol-5-yl)	406.45	$C_{21}H_{22}N_6O_3$	140–145	2933, 1655, 1612, 1498, 1396, 1346, 1253, 1076 and 1010
 (18)	**18b**	2-Cl	341.76	$C_{16}H_{12}ClN_5O_2$	190–192	1618, 1606, 1566, 1498, 1436, 1369, 1249, 1174 and 1033
	18c	3-Cl	341.76	$C_{16}H_{12}ClN_5O_2$	205–207	1618, 1606, 1566, 1498, 1436, 1369, 1249, 1174 and 1033
	18d	3-OMe	337.34	$C_{17}H_{15}N_5O_3$	185–187	1618, 1606, 1596, 1498, 1431, 1369, 1253, 1145 and 1050
	18e	4-OMe	337.34	$C_{17}H_{15}N_5O_3$	211–213	1606, 1573, 1434, 1369, 1315, 1184, 1147, 1087 and 1026
	18f	3-Me	321.34	$C_{17}H_{15}N_5O_2$	221–223	1619, 1596, 1502, 1469, 1419, 1378, 1245, 1176 and 1030
	18g	4-Me	321.34	$C_{17}H_{15}N_5O_2$	218–220	1616, 1500, 1463, 1436, 1365, 1278, 1249, 1184 and 1029

(*continued on next page*)

Table 1 (*continued*)

Structures	No.	X	Mol. wt.	Mol. formula	Mp (°C)	IR (KBr)cm^{-1}
 (19)	**19b**	2-Cl	315.76	$C_{16}H_{14}ClN_3O_2$	210–212 (dec.)	3315, 3138, 1654, 1569, 1382, 1284, 1242, 1118 and 1028
	19c	3-Cl	315.76	$C_{16}H_{14}ClN_3O_2$	254–256 (dec.)	3310, 3125, 1635, 1616, 1596, 1310, 1236, 1165 and 1022
	19d	3-OMe	311.34	$C_{17}H_{17}N_3O_3$	240–243 (dec.)	3350, 3228, 1662, 1575, 1508, 1284, 1247, 1107 and 1039
	19e	4-OMe	311.34	$C_{17}H_{17}N_3O_3$	240–241 (dec.)	3477, 3377, 1635, 1552, 1433, 1330, 1236, 1180 and 1022
	19f	3-Me	295.34	$C_{17}H_{17}N_3O_2$	218–220 (dec.)	3388, 3268, 1650, 1558, 1486, 1288, 1234, 1176 and 1019
	19g	4-Me	295.34	$C_{17}H_{17}N_3O_2$	200–202 (dec.)	3477, 3377, 1641, 1558, 1510, 1322, 1257, 1170 and 1022
 (22)	**22a**	4-NO_2	341.33	$C_{16}H_{15}N_5O_4$	266–268 (dec.)	3450, 3353, 1676, 1533, 1340, 1278, 1236, 1184 and 1029
	22b		465.53	$C_{23}H_{23}N_5O_4S$	264–268 (dec.)	3345, 3254, 1658, 1577, 1365, 1270, 1242, 1149 and 1018

253–255 °C (dec.); IR (KBr): 3336, 1656, 1637, 1614, 1529 1259, 1074 and 1030 cm^{-1}.

*4.1.4. 4,5-Dimethoxy-2-(3-methoxybenzamido)benzamide (**3d**)*

Following a procedure identical to that of **3b**, but using 3-anisic acid (**1d**) (0.6 g, 3.93 mmol), compound **3d** was obtained as a white solid (0.56 g, 48%): mp 207–210 °C (dec.); IR (KBr): 3336, 3193, 1658, 1633, 1614, 1533, 1483, 1278 and 1030 cm^{-1}; ^{1}H NMR: 13.11 (s, 1H, N–*H*), 8.64 (s, 1H, Ar–*H*), 8.01 (b, 1H, N–*H*), 7.61–7.59 (m, 2H, Ar–*H*), 7.42–7.38 (m, 2H, Ar–*H*), 7.09–7.07 (m, 1H, Ar–*H*), 6.52 (b, 1H, N–*H*), 3.98 (s, 3H, O–C*H*$_3$), 3.93 (s, 3H, O–C*H*$_3$) and 3.88 (s, 3H, O–C*H*$_3$).

*4.1.5. 4,5-Dimethoxy-2-(4-methoxybenzamido)benzamide (**3e**)*

Following a procedure identical to that of **3b** but using 4-anisic acid (**1e**) (0.6 g, 3.93 mmol), compound **3e** was obtained as a pale yellow solid (0.60 g, 51%): mp 215–218 °C (dec.); IR (KBr): 3361, 3246, 1670, 1627, 1602, 1525, 1258, 1072 and 1037 cm^{-1}.

*4.1.6. 4,5-Dimethoxy-2-(3-methylbenzamido)benzamide (**3f**)*

Following a procedure identical to that of **3b** but using 3-toluic acid (**1f**) (0.53 g, 3.93 mmol), compound **3f** was obtained as a white solid (0.48 g, 43%): mp 230–235 °C (dec.); IR (KBr): 3417, 3336, 1656, 1635, 1616, 1533, 1377, 1261 and 1041 cm^{-1}.

*4.1.7. 4,5-Dimethoxy-2-(4-methylbenzamido)benzamide (**3g**)*

Following a procedure identical to that of **3b**, but using 4-toluic acid (**1g**) (0.53 g, 3.93 mmol), compound **3g** was obtained as a pale yellow solid (0.63 g, 56%): mp 246–248 °C (dec.); IR (KBr): 3452, 3365, 1666, 1639, 1479, 1386, 1267, 1086 and 1030 cm^{-1}; ^{1}H NMR: 13.22 (s, 1H, N–*H*), 8.60 (s, 1H, Ar–*H*), 8.20 (b, 1H, N–*H*), 7.90–7.88 (d, 2H, Ar–*H*; $J = 8.2$ Hz), 7.44 (s, 1H, Ar–*H*), 7.31–7.29 (d, 2H, Ar–*H*; $J = 8.0$ Hz), 7.25 (b, 1H, N–*H*), 3.93 (s, 3H, O–C*H*$_3$), 3.89 (s, 3H, O–C*H*$_3$) and 2.43 (s, 3H, C*H*$_3$).

*4.1.8. N-[3-(3,4-Dihydro-6,7-dimethoxy-4-oxoquinazolin-2-yl) phenyl]acetamide (**4a**)*

Compound **3a** (0.5 g, 1.47 mmol) was taken in an empty thermometer pocket and fused in a boiling digol bath for 1 h. The solid obtained upon cooling the reaction mixture was triturated with ethyl acetate (10 mL) and filtered. The residue thus obtained was passed through a purifying column of silica (100–190 mesh) using 2% methanol in chloroform as eluent. Compound **4a** was obtained as a white solid (0.14 g, 30%): FAB-MS: $m/z = 340.1$ ($M + H^+$). Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found C, 63.92; H, 5.31; N, 12.52%.

Table 2
Cytotoxicity data of tested compounds.

Compd	% of cells killed at 10 μM			Compd	% of cells killed at 10 μM		
	HCT116 p53$^{+/+}$	HCT116 p53$^{-/-}$	HEY		HCT116 p53$^{+/+}$	HCT116 p53$^{-/-}$	HEY
4a	16	22	12	**18b**	50	63	43
4c	35	37	42	**18c**	56	45	35
4e	10	3	2	**18d**	25	24	13
7a	59	50	35	**18e**	58	48	42
7b	63	57	49	**18f**	55	71	42
7c	80	79	66	**18g**	47	66	29
11a	30	8	0.5	**19b**	18	–	4
11b	34	31	23	**19c**	19	2	3
11c	28	14	9	**19d**	19	20	7
11d	38	22	19	**19e**	21	42	17
16a	6	4	0.5	**19f**	20	27	6
16b	26	27	9	**19g**	5	16	2
16c	19	17	6	**22a**	42	22	4
16f	15	8	2	**22b**	95	95	94
16g	13	3	4				

Table 3
Cytotoxic concentration of select compounds in HCT116 p53$^{+/+}$ and HCT116 p53$^{-/-}$colon cancer cell lines.

Compd	IC_{50} (μM)		Compd	IC_{50} (μM)	
	HCT116 p53$^{+/+}$	HCT116 p53$^{-/-}$		HCT116 p53$^{+/+}$	HCT116 p53$^{-/-}$
7a	9 ± 0.6	>10	**18e**	≈10	7 ± 3
7b	9 ± 1	>10	**18f**	9 ± 1	>10
7c	1.7 ± 1	0.7 ± 0.2	**18g**	8 ± 0.3	9 ± 0.7
18b	8 ± 0.8	>10	**22b**	6 ± 0.05	5 ± 0.3
18c	≈10	≈10			

Cytotoxic concentration (IC_{50}) is defined as drug concentration causing a 50% decrease in cell population using MTT assay as described in the Experimental section.

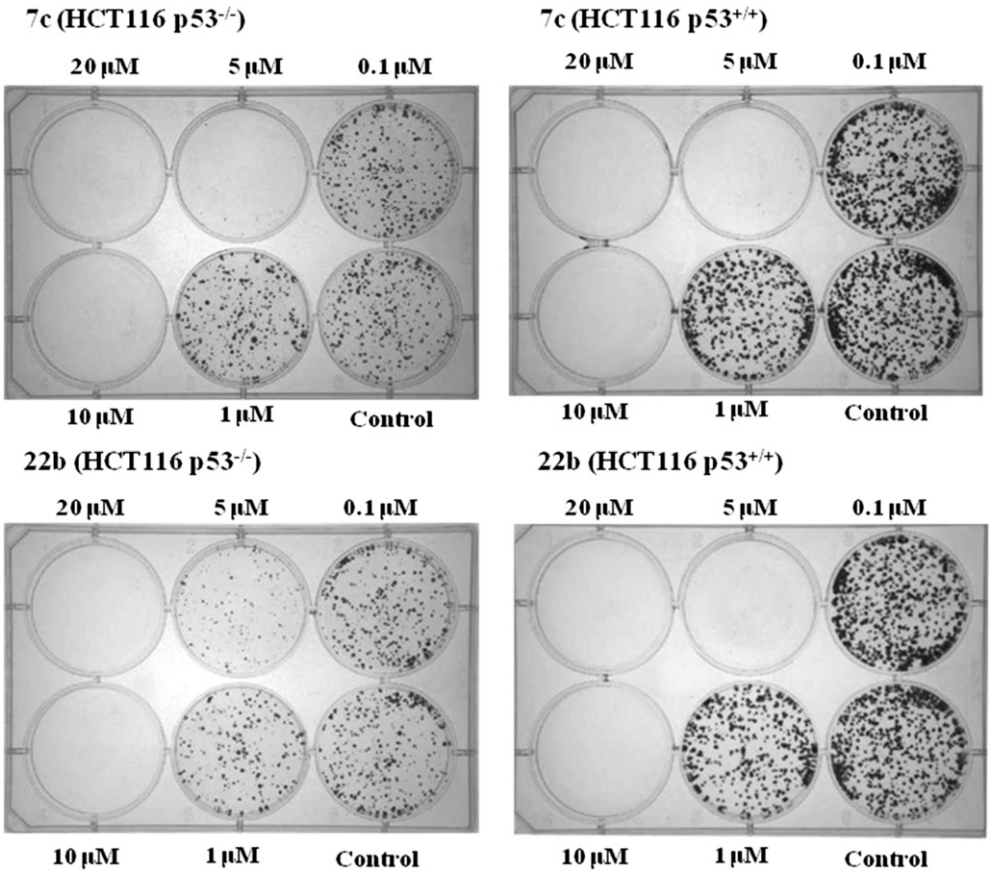


Fig. 2. Treatment of colon cancer cell lines with compounds **7c** and **22b** resulted in a significant inhibition of colony formation. At doses above 5 and 1 μM respectively, compounds **7c** and **22b** abolished cell growth.

*4.1.9. 6,7-Dimethoxy-2-(2-chlorophenyl)-quinazolin-4(3H)-one (**4b**)*

A mixture of diamide **3b** (0.8 g, 2.38 mmol), methanol (5 mL) and aq sodium hydroxide solution (50%, 0.5 mL) was refluxed for 2 h. Excess of methanol was recovered and the residue was quenched in cold water (10 mL), and neutralized with dilute hydrochloric acid (5%). The precipitate formed was filtered, washed with water and dried. The residue thus obtained was recrystallized from chloroform methanol mixture to afford compound **4b** as a solid (0.5 g, 58%): ^{1}H NMR: δ 7.55–7.05 (m, 7H, Ar–*H* and N–*H*), 3.97 (s, 6H, O–C*H*$_3$); FAB-MS: $m/z = 317.2$ ($M + H^+$). Anal. Calcd. for $C_{16}H_{13}ClN_2O_3$: C, 60.67; H, 4.14; N, 8.84. Found C, 60.80; H, 4.30; N, 9.15%.

*4.1.10. 2-(3-Chlorophenyl)-6,7-dimethoxyquinazolin-4(3H)-one (**4c**)*

Following a procedure identical to the one as described for **4a**, but using diamide **3c** (0.50 g, 1.52 mmol), compound **4c** was obtained as a pale yellow solid (0.19 g, 43%): ^{1}H NMR: δ 12.41 (s, 1H, N–*H*), 8.25 (m, 1H, Ar–*H*), 8.15 (m, 1H, Ar–*H*), 7.56 (s, 1H, Ar–*H*), 7.50 (m, 2H, Ar–*H*), 7.22 (s, 1H, Ar–*H*), 4.00 (s, 3H, O–C*H*$_3$) and 3.97 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = (M + H^+)$: 317.8. Anal. Calcd. for $C_{16}H_{13}ClN_2O_3$: C, 60.67; H, 4.14; N, 8.84. Found C, 60.32; H, 4.50; N, 9.04%.

*4.1.11. 6,7-Dimethoxy-2-(3-methoxyphenyl)quinazolin-4(3H)-one (**4d**)*

Following a procedure identical to that of **4b**, but using diamide **3d** (0.50 g, 1.51 mmol), compound **4d** was obtained as a pale yellow solid (0.3 g, 64%): ^{1}H NMR: 12.22 (s, 1H, N–*H*), 7.79–7.76 (m, 2H, Ar–*H*), 7.58 (s, 1H, Ar–*H*), 7.42–7.38 (m, 1H, Ar–*H*), 7.21 (s, 1H, Ar–*H*), 7.06–7.04 (m, 1H, Ar–*H*), 4.04 (s, 3H, O–C*H*$_3$), 4.01 (s, 3H, O–C*H*$_3$) and 3.91 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 312$ (M^+).

*4.1.12. 6,7-Dimethoxy-2-(4-methoxyphenyl)quinazolin-4(3H)-one (**4e**)*

A mixture of diamide **3e** (0.5 g, 1.51 mmol) and freshly prepared sodium *t*-butoxide (0.43 g, 4.53 mmol) in ethylene glycol (1 mL) was exposed to microwave irradiations (300 W, 190 °C) for 1 min. The cooled reaction mixture was quenched in cold water (30 mL) and neutralized with dilute hydrochloric acid (10%). The precipitate so obtained was filtered, washed with cold water and dried to give compound **4e** as a white solid (0.43 g, 90%): ^{1}H NMR: 12.90 (s, 1H, N–*H*), 8.64 (s, 1H, Ar–*H*), 8.01–7.99 (m, 2H, Ar–*H*), 7.34 (s, 1H, Ar–*H*), 7.00–6.97 (m, 2H, Ar–*H*), 3.99 (s, 3H, O–C*H*$_3$), 3.93 (s, 3H, O–C*H*$_3$) and 3.88 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 312.1$ (M^+). Anal. Calcd. for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found C, 65.70; H, 5.20; N, 8.82%.

*4.1.13. 6,7-Dimethoxy-2-m-tolylquinazolin-4(3H)-one (**4f**)*

Following a procedure identical to that of **4b**, but using diamide **3f** (0.7 g, 2.23 mmol), compound **4f** was obtained as a pale yellow solid (0.5 g, 77.0%): ^{1}H NMR: 12.18 (s, 1H, N–*H*), 7.75 (m, 2H, Ar–*H*), 7.44 (s, 1H, Ar–*H*), 7.40 (m, 1H, Ar–*H*), 7.09 (s, 1H, Ar–*H*), 6.96 (m, 1H, Ar–*H*) 4.02 (s, 3H, O–C*H*$_3$), 3.90 (s, 3H, O–C*H*$_3$) and 2.20 (s, 3H, C*H*$_3$); FAB-MS: $m/z = 297.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found C, 68.70; H, 5.20; N, 9.40%.

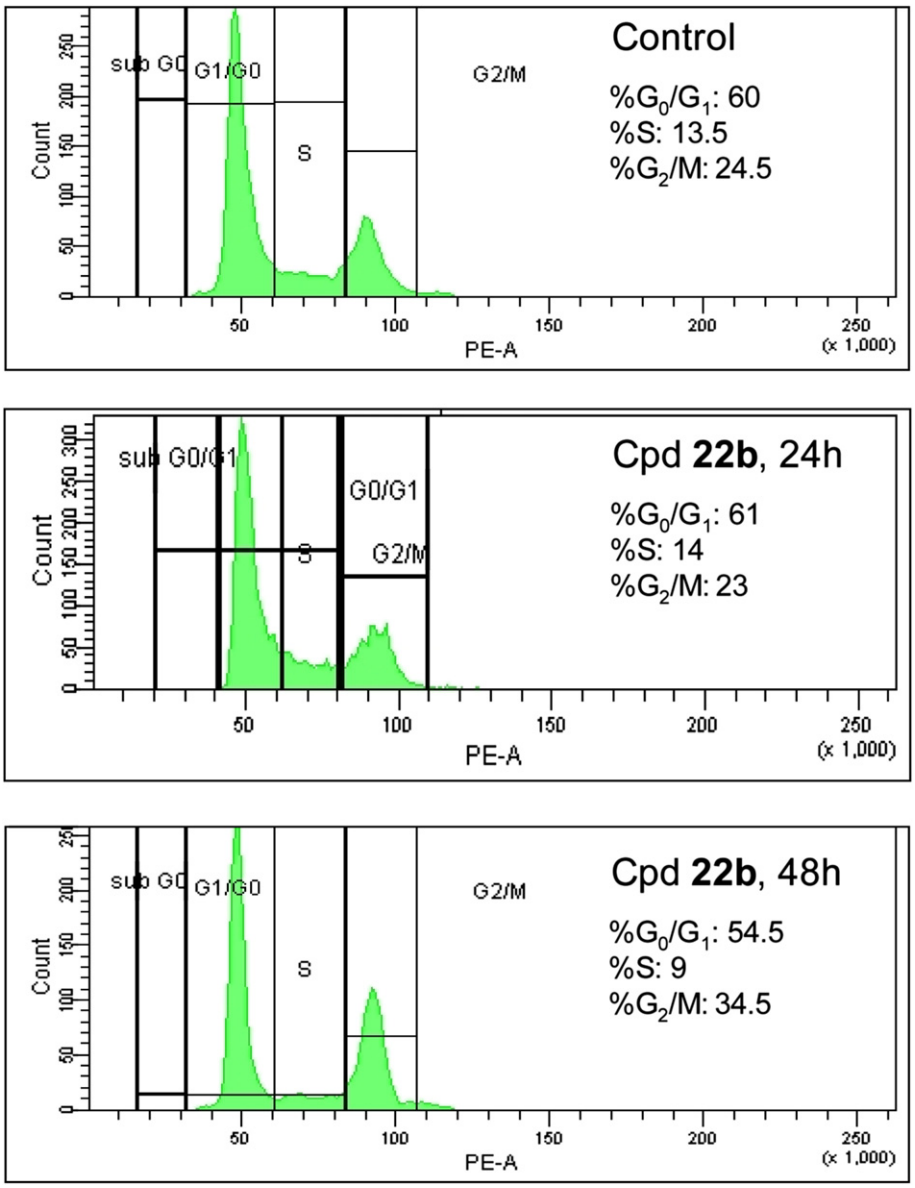


Fig. 3. Flow cytometric analysis of the cell cycle profiles of HCT116 $p53^{-/-}$cells treated with compound **22b**. HCT116 $p53^{-/-}$cells were treated for 24 and 48 h with 1 μM of **22b**. Cell cycle arrest in G2/M phase was observed. Control cells shown were measured at 24 h and no significant changes were observed.

*4.1.14. 6,7-Dimethoxy-2-p-tolylquinazolin-4(3H)-one (**4g**)*

Following a procedure identical to that of **4b**, but using diamide **3g** (0.5 g, 1.59 mmol), compound **4g** was obtained as a pale yellow solid (0.3 g, 64%): ^{1}H NMR: 12.09 (s, 1H, N–*H*), 8.09–8.07 (d, 2H, Ar–*H*; *J* = 8.3 Hz), 7.58 (s, 1H, Ar–*H*), 7.32–7.30 (d, 2H, Ar–*H*; *J* = 8.0 Hz), 7.19 (s, 1H, Ar–*H*), 4.01 (s, 3H, O–C*H*$_3$), 3.98 (s, 3H, O–C*H*$_3$) and 2.43 (s, 3H, C*H*$_3$); FAB-MS: *m*/*z* = 297.1 (M + H$^+$). Anal. Calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found C, 68.81; H, 5.56; N, 9.62%.

*4.1.15. 2-[2-(2-Chlorophenyl)acetamido]-4,5-dimethoxybenzamide (**6a**)*

Following a procedure identical to the one as described for **3b**, but using compound **2** (0.7 g, 3.57 mmol) and 2-chlorophenylacetic acid (**5a**) (0.7 g, 3.93 mmol) as starting materials, compound **6a** was obtained as a white solid (0.2 g, 16%): mp 190–195 °C (dec.); IR (KBr): 3394, 3276, 1679, 1654, 1618, 1525, 1386, 1271 and 1040 cm^{-1}; ^{1}H NMR: 12.19 (s, 1H, N–*H*), 8.39 (s, 1H, Ar–*H*), 7.96 (b, 1H, N–*H*), 7.42–7.38 (m, 2H, Ar–*H*), 7.29–7.24 (m, 3H, Ar–*H*), 6.75 (b, 1H, N–*H*), 3.88 (s, 3H, O–C*H*$_3$), 3.87 (s, 3H, O–C*H*$_3$) and 3.86 (s, 2H, C*H*$_2$); FAB-MS: *m*/*z* = 349.8 (M + H$^+$).

*4.1.16. 2-[2-(4-Chlorophenyl)acetamido]-4,5-dimethoxybenzamide (**6b**)*

Following a procedure identical to that of **3b**, but using compound **2** (0.7 g, 3.57 mmol) and 4-chlorophenylacetic acid (**5b**) (0.66 g, 3.57 mmol) as starting materials, compound **6b** was obtained as a white solid (0.3 g, 19%): mp 228–230 °C (dec.); IR (KBr): 3382, 3311, 1685, 1645, 1614, 1521, 1265, 1087 and 1039 cm^{-1}; ^{1}H NMR: 12.31 (s, 1H, N–*H*), 8.34 (s, 1H, Ar–*H*), 8.04 (b, 1H, N–*H*), 7.88–7.84 (m, 3H, Ar–*H*), 7.35–7.31 (m, 2H, Ar–*H*), 7.10 (b, 1H, N–*H*), 3.86 (s, 3H, O–C*H*$_3$), 3.85 (s, 3H, O–C*H*$_3$) and 3.67 (s, 2H, C*H*$_2$); FAB-MS: *m*/*z* = 349.7 (M + H$^+$).

*4.1.17. 2-[2-(Naphthalen-1-yl)acetamido]-4,5-dimethoxybenzamide (**6c**)*

Following a procedure identical to that of **3b**, but using compound **2** (0.7 g, 3.57 mmol) and 1-naphthylacetic acid (**5c**)

(1.00 g, 3.57 mmol) as starting materials, compound **6c** was obtained as a white solid (1.3 g, 70%): mp 183–185 °C (dec.); IR (KBr): 3470, 3224, 1660, 1619, 1525, 1386, 1271, 1085 and 1037 cm^{-1}; ^1H NMR: 11.38 (s, 1H, N–*H*), 8.41 (s, 1H, Ar–*H*), 8.08–8.01 (m, 1H, Ar–*H*), 7.88–7.83 (m, 2H, Ar–*H*), 7.55–7.45 (m, 4H, Ar–*H*), 6.83 (s, 1H, Ar–*H*), 5.62 (s, 2H, N–*H*), 4.19 (s, 2H, CH_2), 3.92 (s, 3H, O–CH_3) and 3.68 (s, 3H, O–CH_3); FAB-MS: $m/z = 365.4$ ($M + H^+$).

4.1.18. 2-(2-Chlorobenzyl)-6,7-dimethoxyquinazolin-4(3H)-one (**7a**)

Following a procedure identical to the one as described for **4e**, but using compound **6a** (0.2 g, 0.57 mmol) as starting material, compound **7a** was obtained as a solid (0.18 g, 95%): ^1H NMR: 11.50 (s, 1H, N–*H*), 7.56 (s, 1H, Ar–*H*), 7.41–7.39 (m, 1H, Ar–*H*), 7.31–7.29 (m, 1H, Ar–*H*), 7.26–7.23 (m, 2H, Ar–*H*), 7.06 (s, 1H, Ar–*H*), 4.15 (s, 2H, CH_2), 4.00 (s, 3H, O–CH_3) and 3.96 (s, 3H, O–CH_3); FAB-MS: $m/z = 331.8$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57; N, 8.47. Found C, 61.62; H, 4.71; N, 8.28%.

4.1.19. 2-(4-Chlorobenzyl)-6,7-dimethoxyquinazolin-4(3H)-one (**7b**)

Following a procedure identical to that of **4e**, but using compound **6b** (0.2 g, 0.57 mmol) as starting material, compound **7b** was obtained as a solid (0.17 g, 89%): ^1H NMR: 11.99 (s, 1H, N–*H*), 7.52 (s, 1H, Ar–*H*), 7.41–7.37 (m, 2H, Ar–*H*), 7.29–7.26 (m, 2H, Ar–*H*), 7.09 (s, 1H, Ar–*H*), 3.98 (s, 3H, O–CH_3), 3.96 (s, 3H, O–CH_3) and 3.94 (s, 2H, CH_2); FAB-MS: $m/z = 331.8$ ($M + H^+$). Anal. Calcd for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57; N, 8.47. Found C, 61.80; H, 4.76; N, 8.60%.

4.1.20. 6,7-Dimethoxy-2-[(naphthalen-1-yl)methyl]quinazolin-4(3H)-one (**7c**)

Following a procedure identical to that of **4e**, but using compound **6c** (0.2 g, 0.55 mmol) as starting material, compound **7c** was obtained as a solid (0.17 g, 93%): ^1H NMR: 11.57 (s, 1H, N–*H*), 8.23–8.21 (m, 1H, Ar–*H*), 7.88–7.86 (d, 1H, Ar–*H*; $J = 8.1$ Hz), 7.81–7.79 (d, 1H, Ar–*H*; $J = 8.0$ Hz), 7.56–7.42 (m, 5H, Ar–*H*), 7.08 (s, 1H, Ar–*H*), 4.47 (s, 2H, CH_2), 3.97 (s, 3H, O–CH_3) and 3.95 (s, 3H, O–CH_3); FAB-MS: $m/z = 347.4$ ($M + H^+$). Anal. Calcd. for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found C, 72.70; H, 5.34; N, 8.26%.

4.1.21. 2-Chloromethyl-6,7-dimethoxyquinazolin-4(3H)-one (**9**)

Chloroacetonitrile (1.17 mL, 18.96 mmol) was added to a saturated solution of dry HCl in dioxane (10 mL). The mixture was stirred at 0–5 °C for 10 min under anhydrous conditions. Compound **8** (2.0 g, 9.48 mmol) was added into the above solution and the reaction mixture was stirred at rt for another 24 h. The mixture was quenched in ice-cold water (150 mL) and neutralized with aq ammonia. The precipitate formed was filtered, washed with cold water and dried to afford compound **9** as a pale yellow solid (1.8 g, 75%): mp 219–223 °C; IR (KBr): 2931, 1670, 1608, 1498, 1438, 1377, 1240, 1078 and 1022 cm^{-1}; FAB-MS: $m/z = 255.7$ ($M + H^+$).

4.1.22. 4-[(3,4-Dihydro-6,7-dimethoxy-4-oxoquinazolin-2-yl) methylamino]benzoic acid (**11a**)

4-Aminobenzoic acid (**10a**) (0.80 g, 5.89 mmol) and potassium carbonate (0.62 g, 5.89 mmol) were added to a solution of compound **9** (0.5 g, 1.96 mmol) in DMF (1 mL). The reaction mixture was stirred at rt for 8 h, quenched into cold water and neutralized (pH 4.5–5.0) with dilute hydrochloric acid. The precipitate formed was filtered, washed with cold water and dried. The solid thus obtained was recrystallized from a mixture of chloroform and methanol to afford compound **11a** (0.24 g, 35%): ^1H NMR: 12.35 (b, 1H, N–*H*), 7.71–7.69 (d, 2H, Ar–*H*; $J = 8.7$ Hz), 7.43 (s, 1H, Ar–*H*), 7.09 (s, 1H, Ar–*H*), 6.58–6.56 (d, 2H, Ar–*H*; $J = 8.7$ Hz), 6.06 (b, 1H, N–*H*), 5.06 (s, 2H, CH_2), 3.87 (s, 3H, O–CH_3) and 3.86 (s, 3H, O–CH_3); FAB-MS: $m/z = 356.3$ ($M + H^+$). Anal. Calcd. for $C_{18}H_{17}N_3O_5$: C, 60.84; H, 4.82; N, 11.82. Found C, 60.60; H, 4.72; N, 11.96%.

4.1.23. 2-[(3-Methanesulfonylamidophenylamino)methyl]-6,7-dimethoxyquinazolin-4(3H)-one (**11b**)

Following a procedure identical to the one as described for **11a**, but using 3-methanesulfonylamidoaniline (**10b**) (1.09 g, 5.89 mmol), compound **11b** was obtained as a solid (0.43 g, 54%): ^1H NMR: 12.26 (b, 1H, N–*H*), 7.40 (s, 1H, Ar–*H*), 7.02 (m, 2H, Ar–*H*), 5.80 (m, 2H, Ar–*H*), 5.59 (m, 1H, Ar–*H*), 5.06 (b, 1H, N–*H*), 4.00 (s, 1H, N–*H*), 3.85 (s, 3H, O–CH_3) and 3.80 (s, 3H, O–CH_3), 3.44 (m, 2H, CH_2), 2.82 (s, 3H, SO_2–CH_3); FAB-MS: $m/z = 405.4$ ($M + H^+$). Anal. Calcd. for $C_{18}H_{20}N_4O_5S$: C, 53.46; H, 4.98; N, 13.85. Found C, 53.42; H, 5.01; N, 13.80%.

4.1.24. 2-[(4-Methanesulfonylamidophenylamino)methyl]-6,7-dimethoxyquinazolin-4(3H)-one (**11c**)

Following a procedure identical to that of **11a**, but using 4-methanesulfonylamidoaniline (**10c**) (1.09 g, 5.89 mmol), compound **11c** was obtained as a solid (0.5 g, 63%): ^1H NMR: 12.30 (b, 1H, N–*H*), 7.40 (s, 1H, Ar–*H*), 7.00 (s, 1H, Ar–*H*), 6.85 (d, 2H, Ar–*H*; $J = 9.0$ Hz), 6.33 (d, 2H, Ar–*H*; $J = 9.0$ Hz), 6.06 (b, 1H, N–*H*), 5.06 (s, 2H, CH_2), 3.87 (s, 3H, O–CH_3) and 3.86 (s, 3H, O–CH_3), 2.77 (s, 3H, SO_2–CH_3); FAB-MS: $m/z = 405.4$ ($M + H^+$). Anal. Calcd. for $C_{18}H_{20}N_4O_5S$: C, 53.46; H, 4.98; N, 13.85. Found C, 53.50; H, 5.06; N, 13.92%.

4.1.25. 2-[(4-Methoxyphenylamino)methyl]-6,7-dimethoxyquinazolin-4(3H)-one (**11d**)

Following a procedure identical to that of **11a**, but using 4-anisidine (**10d**) (0.72 g, 5.89 mmol), compound **11d** was obtained as a solid (0.42 g, 64%): ^1H NMR: 12.30 (b, 1H, N–*H*), 7.48 (s, 1H, Ar–*H*), 7.45–7.42 (m, 2H, Ar–*H*), 7.19 (s, 1H, Ar–*H*), 7.14–7.00 (m, 2H, Ar–*H*), 6.26 (b, 1H, N–*H*), 4.82 (s, 2H, CH_2), 3.97 (s, 3H, O–CH_3), 3.88 (s, 3H, O–CH_3) and 3.87 (s, 3H, O–CH_3); FAB-MS: $m/z = 342.4$ ($M + H^+$). Anal. Calcd. for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31. Found C, 63.56; H, 5.76; N, 12.57%.

4.1.26. N-n-Butyl-4,5-dimethoxy-2-nitrobenzamide (**13**)

A mixture of 4,5-dimethoxy-2-nitrobenzoic acid (**12**) (5.0 g, 22.0 mmol) and thionyl chloride (15 mL) was refluxed under anhydrous conditions for 2 h. Excess of thionyl chloride was recovered under reduced pressure and the residue was dissolved in anhydrous THF (10 mL). This was then added to a solution of *n*-butylamine (2.6 mL, 26.4 mmol) and triethylamine (TEA, 9.2 mL, 66.0 mmol) in THF (15 mL) at 0–5 °C. The reaction mixture was stirred for 2 h at rt and quenched in cold water. The precipitate so formed was filtered, washed with cold water and dried. The solid thus obtained was crystallized from methanol to obtain yellow crystals (4.8 g, 77%): mp 130–132 °C; IR (KBr): 3269, 1639, 1519, 1340, 1278, 1224, 1180 and 1026 cm^{-1}.

4.1.27. 2-Amino-N-n-butyl-4,5-dimethoxybenzamide (**14**)

A solution of 2-nitro-*N*-*n*-butyl-4,5-dimethoxybenzamide (**13**) (1.0 g, 3.5 mmol) in methanol (20 mL) was refluxed on a water bath. Iron powder (1.6 g, 28.4 mmol) and a solution of ammonium chloride (1.5 g, 28.3 mmol) in water (2 mL) were added portion-wise (in 4 parts at an interval of 45 min) to the above refluxing solution. Refluxing was continued for 7–8 h and the solution was filtered through filtering aid and washed with hot methanol (2 × 10 mL). The filtrate was concentrated under

reduced pressure to remove excess of methanol and the resulting aqueous solution was diluted with water (25 mL), basified with sodium bicarbonate (10% aq solution) and extracted with chloroform (3 × 50 mL). The combined chloroform layer was dried and concentrated to get brown colored residue which was dried under vacuum to afford compound **14** (0.84 g, 95%): mp 109–111 °C; IR (KBr): 3409, 3317, 1635, 1508, 1463, 1373, 1261, 1180 and 1026 cm^{-1}.

4.1.28. 4,5-Dimethoxy-N-n-butyl-2-{[4-((methylsulfonyl)amino) phenyl]carboxamido}benzamide (**15a**)

Following a procedure identical to the one as described for **3b**, but using compound **14** (0.5 g, 1.98 mmol) and 4-methanesulfonamidobenzoic acid (**1a**) (0.47 g, 2.18 mmol), compound **15a** was obtained as a pale yellow solid (0.49 g, 55%) and used in the next step without further purification: mp 184–187 °C; IR (KBr): 3460, 1656, 1633, 1614, 1445, 1338, 1245, 1155 and 1010 cm^{-1}.

4.1.29. 4,5-Dimethoxy-N-n-butyl-2-{[3-((methylsulfonyl)amino) phenyl]carboxamido}benzamide (**15b**)

Following a procedure identical to that of **3b**, but using compound **14** (0.5 g, 1.98 mmol) and 3-methanesulfonamidobenzoic acid (**1b**) (0.47 g, 2.18 mmol), compound **15b** was obtained as a pale yellow solid (0.47 g, 54%) and used in the next step without further purification: mp 221–223 °C; IR (KBr): 3390, 1663, 1640, 1611, 1486, 1332, 1257, 1149 and 1038 cm^{-1}.

4.1.30. 2-{[(4-(Acetamido)phenyl)carbonyl]amino}-4,5-Dimethoxy-N-n-butylbenzamide (**15c**)

Following a procedure identical to that of **3a**, but using compound **14** (0.5 g, 1.98 mmol) and 4-acetamidobenzoic acid (**1c**) (0.39 g, 2.18 mmol), compound **15c** was obtained as a white solid (0.47 g, 58%) and used in the next step without further purification: mp 115–117 °C; IR (KBr): 3272, 1658, 1643, 1539, 1424, 1322, 1242, 1166 and 1019 cm^{-1}.

4.1.31. 2-[4-(Cyanophenyl)carboxamido]-4,5-dimethoxy-N-n-butylbenzamide (**15d**)

Following a procedure identical to that of **3b**, but using compound **14** (0.5 g, 1.98 mmol) and 4-cyanobenzoic acid (**1d**) (0.32 g, 2.18 mmol), compound **15d** was obtained as a white solid (0.52 g, 70%) and used in the next step without further purification: mp 190–193 °C; IR (KBr): 3428, 3330, 2233, 1665, 1647, 1618, 1251, 1080 and 1024 cm^{-1}.

4.1.32. 2-[3-(Cyanophenyl)carboxamido]-4,5-dimethoxy-N-n-butylbenzamide (**15e**)

Following a procedure identical to that of **3b**, but using compound **14** (0.5 g, 1.98 mmol) and 3-cyanobenzoic acid (**1e**) (0.32 g, 2.18 mmol), compound **15e** was obtained as a white solid (0.49 g, 66%) and used in the next step without further purification: mp 184–186 °C; IR (KBr,): 3430, 3345, 2222, 1660, 1640, 1616, 1247, 1076 and 1030 cm^{-1}.

4.1.33. 4,5-Dimethoxy-N-n-butyl-2-{[4-(1H-tetrazol-5-yl)phenyl] carboxamido}benzamide (**15f**)

Sodium azide (0.3 g, 4.88 mmol) and ammonium chloride (0.4 g, 8.14 mmol) were added to the stirred solution of compound **15d** (0.5 g, 1.31 mmol) in DMF (1 mL). The reaction mixture was heated at 100 °C with stirring for 6 h. The reaction mixture was quenched into cold water and acidified with dilute hydrochloric acid (5%). The precipitate so formed was filtered, washed with water and dried. Pure compound **15f** was so obtained as white crystals (0.38 g, 68%): mp 220–223 °C; IR (KBr): 3359, 1670, 1635, 1596, 1498, 1348, 1269, 1176 and 1010 cm^{-1}; ^{1}H NMR: 12.72 (b, 1H, N–*H*), 8.58 (s, 1H, Ar–*H*), 8.14 (d, 2H, Ar–*H*), 7.80 (d, 2H, Ar–*H*), 6.92 (s, 1H, Ar–*H*), 6.10 (b, 1H, N–*H*), 4.01 (s, 3H, O–C*H*$_3$), 3.92 (s, 3H, O–C*H*$_3$), 3.47 (m, 2H, N–C*H*$_2$), 1.60 (m, 2H, C*H*$_2$), 1.40 (m, 2H, C*H*$_2$) and 0.98 (t, 3H, C*H*$_3$); FAB-MS: $m/z = 425.4$ ($M + H^+$).

4.1.34. 4,5-Dimethoxy-N-n-butyl-2-{[3-(1H-tetrazol-5-yl)phenyl] carboxamido}benzamide (**15g**)

Following a procedure identical to the one as described for**15f**, but using compound **15e** (0.50 g, 1.31 mmol), compound **15g** was obtained as a white solid (0.41 g, 74%) and used in the next step without further purification: mp 140–145 °C; IR (KBr): 3334, 1666, 1635, 1596, 1488, 1356, 1255, 1178 and 1025 cm^{-1}; ^{1}H NMR: 12.87 (s, 1H, N–*H*), 8.75 (s, 1H, Ar–*H*), 8.58 (m, 1H, Ar–*H*), 8.33–8.31 (d, 1H, Ar–*H*; $J = 7.8$ Hz), 8.16–8.14 (d, 1H, Ar–*H*; $J = 7.8$ Hz), 7.95 (b, 1H, N–*H*), 7.71–7.67 (m, 1H, Ar–*H*), 7.27 (s, 1H, Ar–*H*), 3.99 (s, 3H, O–C*H*$_3$), 3.95 (s, 3H, O–C*H*$_3$), 3.44–3.39 (m, 2H, C*H*$_2$), 1.65–1.60 (m, 2H, C*H*$_2$), 1.45–1.40 (m, 2H, C*H*$_2$) and 0.98–0.95 (t, 3H, C*H*$_3$); FAB-MS: $m/z = 425.4$ ($M + H^+$).

4.1.35. N-[4-(6,7-Dimethoxy-3-n-butyl-4-oxo-3,4-dihydroquinazolin-2-yl)phenyl]methanesulfonamide (**16a**)

Following a procedure identical to that of **4b**, but using compound **15a** (0.5 g, 1.11 mmol), compound **16a** was obtained as a white solid (0.37 g, 78%): ^{1}H NMR: 7.75 (s, 1H, Ar–*H*), 7.50 (m, 1H, Ar–*H*), 7.37 (m, 3H, Ar–*H*), 7.17 (m, 1H, Ar–*H*), 4.00 (m, 8H, 6H- 2× O–C*H*$_3$ and 2H- C*H*$_2$), 3.05 (s, 3H, SO_2CH_3), 1.25 (m, 4H, 2× C*H*$_2$) and 0.80 (m, 3H, C*H*$_3$); FAB-MS: $m/z = 432.5$ ($M + H^+$). Anal. Calcd. for $C_{21}H_{25}N_3O_5S$: C, 58.45; H, 5.84; N, 9.74. Found C, 58.01; H, 5.67; N, 9.88%.

4.1.36. N-[3-(6,7-Dimethoxy-3-n-butyl-4-oxo-3,4-dihydroquinazolin-2-yl)phenyl]methanesulfonamide(**16b**)

Following a procedure identical to that of **4b**, but using compound **15b** (0.5 g, 1.11 mmol), compound **16b** was obtained as a white solid (0.43 g, 82%): ^{1}H NMR: 7.67 (s, 1H, Ar–*H*), 7.54–7.52 (m, 1H, Ar–*H*), 7.42–7.39 (m, 2H, Ar–*H*), 7.18–7.16 (m, 1H, Ar–*H*), 6.78 (s, 1H, Ar–*H*), 4.02–3.99 (m, 8H, 6H- 2× O–C*H*$_3$ and 2H- C*H*$_2$), 3.09 (s, 3H, C*H*$_3$), 1.30–1.18 (m, 4H, C*H*$_2$) and 0.82–0.78 (m, 3H, C*H*$_3$); FAB-MS: $m/z = 432.5$ ($M + H^+$). Anal. Calcd. for $C_{21}H_{25}N_3O_5S$: C, 58.45; H, 5.84; N, 9.74. Found C, 58.63; H, 6.06; N, 10.03%.

4.1.37. N-[4-(6,7-Dimethoxy-3-n-butyl-4-oxo-3,4-dihydroquinazolin-2-yl)phenyl]acetamide (**16c**)

Following a procedure identical to that of **4e**, but using compound **15c** (0.5 g, 1.21 mmol), compound **16c** was obtained as a white solid (0.33 g, 69%): ^{1}H NMR: 7.75 (m, 2H, Ar–*H*), 7.60 (m, 2H, Ar–*H*), 7.25 (m, 1H, Ar–*H*), 6.80 (s, 1H, Ar–*H*), 4.00 (m, 6H, 2× O–C*H*$_3$), 3.15 (m, 2H, C*H*$_2$), 2.35 (s, 3H, C*H*$_3$), 1.60 (m, 2H, C*H*$_2$), 1.30 (m, 2H, C*H*$_2$) and 1.05 (m, 3H, C*H*$_3$); FAB-MS: $m/z = 396.4$. Anal. Calcd. for $C_{22}H_{25}N_3O_4$: C, 66.81; H, 6.37; N, 10.63. Found C, 63.08; H, 6.12; N, 10.22%.

4.1.38. 6,7-Dimethoxy-3-butyl-2-[4-(1H-tetrazol-5-yl)phenyl] quinazoline-4(3H)-one (**16f**)

Following a procedure identical to that of **4e**, but using compound **15f** (0.5 g, 1.31 mmol), compound **16f** was obtained as a white solid (0.38 g, 62%): ^{1}H NMR: 7.88–7.86 (d, 2H, Ar–*H*; $J = 8.1$ Hz), 7.71 (s, 1H, Ar–*H*), 7.58–7.56 (d, 2H, Ar–*H*; $J = 8.2$ Hz), 7.44 (s, 1H, Ar–*H*), 4.07 (s, 3H, O–C*H*$_3$), 4.03 (s, 3H, O–C*H*$_3$), 3.99–3.97 (m, 2H, C*H*$_2$), 1.61–1.57 (m, 2H, C*H*$_2$), 1.19–1.13 (m, 2H, C*H*$_2$) and 0.78–0.74 (t, 3H, C*H*$_3$); FAB-MS: $m/z = 407.4$ ($M + H^+$). Anal. Calcd. for $C_{21}H_{22}N_6O_3$: C, 62.06; H, 5.46; N, 20.68. Found C, 62.42; H, 5.21; N, 20.28%.

*4.1.39. 6,7-Dimethoxy-3-n-butyl-2-[3-(1H-tetrazol-5-yl)phenyl] quinazoline-4(3H)-one (**16g**)*

Following a procedure identical to that of **4e**, but using compound **15g** (0.5 g, 1.31 mmol), compound **16g** was obtained as a white solid (0.41 g, 74%): ^{1}H NMR: 7.84 (s, 1H, Ar–*H*), 7.45 (m, 3H, Ar–*H*), 7.30 (s, 1H, Ar–*H*), 6.95 (s, 1H, Ar–*H*), 4.00 (s, 3H, O–C*H*$_3$), 3.85 (s, 3H, O–C*H*$_3$), 3.65 (m, 2H, C*H*$_2$), 1.40 (m, 2H, C*H*$_2$), 1.33 (m, 2H, C*H*$_2$) and 0.85 (m, 3H, C*H*$_3$); FAB-MS: $m/z = 407.4$ ($M + H^+$). Anal. Calcd. for $C_{21}H_{22}N_6O_3$: C, 62.06; H, 5.46; N, 20.68. Found C, 62.31; H, 5.38; N, 20.32%.

*4.1.40. General procedure for the synthesis of chloro derivatives (**17b**–**17g**)*

The synthesis of 4-chloro-2-(2-chlorophenyl)-6,7-dimethoxyquinazoline (**17b**) is reported as a representative example.

A mixture of compound **4b** (0.2 g, 0.63 mmol), phosphorus oxychloride (1 mL) and one drop of DMF was refluxed with stirring on an oil bath for 8 h. The reaction mixture was cooled to rt and quenched into crushed ice. The solid obtained was filtered, washed with cold water and immediately dissolved in chloroform. The resulting solution was dried over sodium sulfate and concentrated to afford compound **17b** as pale yellow solid (0.19 g, 89%), which was used in the subsequent step without further purification: mp 188–190 °C; IR (KBr): 1612, 1556, 1502, 1444, 1406, 1352, 1236, 1161 and 1031 cm^{-1}.

*4.1.40.1. 4-Chloro-2-(3-chlorophenyl)-6,7-dimethoxyquinazoline (**17c**).* (0.18 g, 85%); mp. 167–170 °C; IR (KBr): 1618, 1554, 1502, 1454, 1404, 1336, 1238, 1164 and 1033 cm^{-1}.

*4.1.40.2. 4-Chloro-6,7-dimethoxy-2-(3-methoxyphenyl)quinazoline (**17d**).* (0.64 g, 90%); mp. 188–190 °C; IR (KBr): 1604, 1560, 1514, 1438, 1402, 1353, 1255, 1170 and 1020 cm^{-1}.

*4.1.40.3. 4-Chloro-6,7-dimethoxy-2-(4-methoxyphenyl)quinazoline (**17e**).* (0.72 g, 85%); mp. 170–172 °C; IR (KBr): 1614, 1562, 1502, 1460, 1404, 1336, 1238, 1161 and 1033 cm^{-1}.

*4.1.40.4. 4-Chloro-6,7-dimethoxy-2-(3-methylphenyl)quinazoline (**17f**).* (0.17 g, 80%); mp. 230–232 °C; IR (KBr): 1612, 1560, 1500, 1438, 1398, 1353, 1253, 1159 and 1020 cm^{-1}.

*4.1.40.5. 4-Chloro-6,7-dimethoxy-2-(4-methylphenyl)quinazoline (**17g**).* (0.18 g, 84%); mp. 188–190 °C; IR (KBr): 1616, 1560, 1498, 1417, 1398, 1353, 1234, 1159 and 1018 cm^{-1}.

*4.1.41. 4-Azido-2-(2-chlorophenyl)-6,7-dimethoxyquinazoline (**18b**)*

Compound **17b** (0.7 g, 2.08 mmol) was dissolved in DMF (1 mL) and added sodium azide (0.41 g, 6.35 mmol). The reaction mixture was exposed to microwave radiations (300 W) at 100 °C for 3 min, cooled to rt and quenched into cold water (25 mL). The precipitate formed was filtered, washed with cold water and dried to afford compound **18b** (0.70 g, 68%): ^{1}H NMR: 8.03 (s, 1H, Ar–*H*), 7.75 (m, 1H, Ar–*H*), 7.62 (m, 3H, Ar–*H*), 7.54 (m, 1H, Ar–*H*), 4.16 (s, 3H, O–C*H*$_3$) and 4.09 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 342.7$ ($M + H^+$). Anal. Calcd. for $C_{16}H_{12}ClN_5O_2$: C, 56.23; H, 3.54; N, 20.49. Found C, 56.36; H, 3.64; N, 20.60%.

*4.1.42. 4-Azido-2-(3-chlorophenyl)-6,7-dimethoxyquinazoline (**18c**)*

Following a procedure identical to the one as described for **18b**, but using compound **17c** (0.7 g, 2.08 mmol), compound **18c** was obtained as a pale yellow solid (0.70 g, 68%): ^{1}H NMR: 8.65 (m, 2H, Ar–*H*), 7.99 (s, 1H, Ar–*H*), 7.60 (m, 3H, Ar–*H*), 4.15 (s, 3H, O–C*H*$_3$) and 4.12 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 342.7$ ($M + H^+$). Anal. Calcd for $C_{16}H_{12}ClN_5O_2$: C, 56.23; H, 3.54; N, 20.49. Found C, 56.48; H, 3.79; N, 20.68%.

*4.1.43. 4-Azido-6,7-dimethoxy-2-(3-methoxyphenyl)quinazoline (**18d**)*

Following a procedure identical to that of **18b**, but using compound **17d** (0.7 g, 2.12 mmol), compound **18d** was obtained as a white solid (0.60 g, 84%): ^{1}H NMR: 8.24 (m, 1H, Ar–*H*), 8.18 (m, 1H, Ar–*H*), 7.99 (s, 1H, Ar–*H*), 7.80 (s, 1H, Ar–*H*), 7.55 (m, 1H, Ar–*H*), 7.21 (m, 1H, Ar–*H*) 4.14 (s, 3H, O–C*H*$_3$), 4.11 (s, 3H, O–C*H*$_3$) and 3.97 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 338.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{15}N_5O_3$:C, 60.53; H, 4.48; N, 20.76. Found C, 60.18; H, 4.62; N, 20.63%.

*4.1.44. 4-Azido-6,7-dimethoxy-2-(4-methoxyphenyl)quinazoline (**18e**)*

Following a procedure identical to that of **18b**, but using compound **17e** (0.7 g, 2.12 mmol), compound **18e** was obtained as a white solid (0.50 g, 70%): ^{1}H NMR: 8.68 (d, 2H, Ar–*H*), 7.96 (s, 1H, Ar–*H*), 7.55 (s, 1H, Ar–*H*), 7.13 (d, 2H, Ar–*H*), 4.12 (s, 3H, O–C*H*$_3$), 4.10 (s, 3H, O–C*H*$_3$) and 3.95 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 338.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{15}N_5O_3$: C, 60.53; H, 4.48; N, 20.76. Found C, 60.22; H, 4.64; N, 20.66%.

*4.1.45. 4-Azido-6,7-dimethoxy-2-m-tolylquinazoline (**18f**)*

Following a procedure identical to that of **18b**, but using compound **17f** (0.17 g, 0.54 mmol), compound **18f** was obtained as a white solid (0.13 g, 75%): ^{1}H NMR: 8.61 (d, 2H, Ar–*H*), 7.92 (s, 1H, Ar–*H*), 7.50 (s, 1H, Ar–*H*), 7.22 (d, 2H, Ar–*H*), 4.08 (s, 3H, O–C*H*$_3$), 4.00 (s, 3H, O–C*H*$_3$) and 3.85 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 322.3$. Anal. Calcd. for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79. Found C, 63.73; H, 4.87; N, 21.52%.

*4.1.46. 4-Azido-6,7-dimethoxy-2-p-tolylquinazoline (**18g**)*

Following a procedure identical to that of **18b**, but using compound **17g** (0.1 g, 0.32 mmol), compound **18g** was obtained as a white solid (0.10 g, 98%): ^{1}H NMR: 8.55 (d, 2H, Ar–*H*), 7.98 (s, 1H, Ar–*H*), 7.58 (s, 1H, Ar–*H*), 7.44 (d, 2H, Ar–*H*), 4.13 (s, 3H, O–C*H*$_3$), 4.10 (s, 3H, O–C*H*$_3$) and 2.51 (s, 3H, Ar–C*H*$_3$); FAB-MS: $m/z = 322.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79. Found C, 63.84; H, 4.87; N, 21.66%.

*4.1.47. 2-(2-Chlorphenyl)-6,7-dimethoxyquinazolin-4-amine (**19b**)*

Compound **18b** (0.12 g, 0.35 mmol) was dissolved in methanol (19 mL) in a three-neck Rb flask (100 mL) equipped with a hydrogen balloon. Palladium–charcoal (0.1 g, 10%) was added to the above solution and the reaction mixture was stirred under the atmosphere of hydrogen gas for 4 h. The reaction mixture was filtered through filtering aid, washed with hot methanol and the filtrate was concentrated under reduced pressure to afford compound **19b** as a pale yellow solid (0.10 g, 90%): ^{1}H NMR: 9.4 (b, 1H, N–*H*), 8.52 (m, 1H, Ar–*H*), 8.20 (b, 2H, N–*H* and Ar–*H*), 7.96 (s, 1H, Ar–*H*), 7.58 (m, 3H, Ar–*H*), 4.09 (s, 3H, O–C*H*$_3$) and 4.06 (s, 3H, O–C*H*$_3$); FAB-MS: $m/z = 316.7$ ($M + H^+$). Anal. Calcd. for $C_{16}H_{14}ClN_3O_2$: C, 60.86; H, 4.47; N, 13.31. Found C, 60.69; H, 4.71; N, 13.63%.

*4.1.48. 2-(3-Chlorphenyl)-6,7-dimethoxyquinazolin-4-amine (**19c**)*

Following a procedure identical to the one as described for **19b**, but using compound **18c** (0.60 g, 1.76 mmol) as starting material, compound **19c** was obtained as a white solid (0.42 g, 76%): ^{1}H NMR: 9.04 (b, 2H, N–H_2), 8.54 (d, 2H, Ar–*H*), 8.30 (b, 1H, Ar–*H*), 7.59 (m, 3H, Ar–*H*), 4.09 (s, 3H, O–C*H*$_3$) and 4.06 (s, 3H, O–C*H*$_3$); FAB-MS:

$m/z = 316.7$ ($M + H^+$). Anal. Calcd. for $C_{16}H_{14}ClN_3O_2$: C, 60.86; H, 4.47; N, 13.31. Found C, 60.77; H, 4.12; N, 12.98%.

4.1.49. 6,7-Dimethoxy-2-(3-methoxyphenyl)quinazolin-4-amine (**19d**)

Following a procedure identical to that of **19b**, but using compound **18d** (0.8 g, 2.37 mmol) as starting material, compound **19d** was obtained as a white solid (0.63 g, 86%): ^{1}H NMR: 8.06–8.02 (m, 2H, Ar–*H*), 7.48 (s, 1H, Ar–*H*), 7.39–7.35 (m, 1H, Ar–*H*), 7.32 (s, 1H, Ar–*H*), 6.99–6.97 (m, 1H, Ar–*H*), 6.66 (b, 2H, N–H_2), 4.04 (s, 3H, O–CH_3), 4.02 (s, 3H, O–CH_3) and 3.92 (s, 3H, O–CH_3); FAB-MS: $m/z = 312.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found C, 65.73; H, 5.58; N, 13.60%.

4.1.50. 6,7-Dimethoxy-2-(4-methoxyphenyl)quinazolin-4-amine (**19e**)

Following a procedure identical to that of **19b**, but using compound **18e** (0.3 g, 0.89 mmol) as starting material, compound **19e** was obtained as a white solid (0.24 g, 88%): ^{1}H NMR: 8.43–8.40 (m, 2H, Ar–*H*), 7.29 (s, 1H, Ar–*H*), 7.01–6.98 (m, 2H, Ar–*H*), 6.91 (s, 1H, Ar–*H*), 5.34 (b, 2H, N–H_2), 4.04 (s, 3H, O–CH_3), 4.00 (s, 3H, O–CH_3) and 3.88 (s, 3H, O–CH_3); FAB-MS: $m/z = 312.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found C, 65.88; H, 5.66; N, 13.67%.

4.1.51. 6,7-Dimethoxy-2-m-tolylquinazolin-4-amine (**19f**)

Following a procedure identical to that of **19b**, but using compound **18f** (0.13 g, 0.39 mmol) as starting material, compound **19f** was obtained as a white solid (0.10 g, 90.0%): ^{1}H NMR: 8.36 (m, 2H, Ar–*H*), 7.50 (m, 2H, Ar–*H*), 7.28 (m, 2H, Ar–*H*), 7.00 (b, 2H, N–H_2), 4.05 (s, 3H, O–CH_3), 4.03 (s, 3H, O–CH_3) and 2.18 (s, 3H, Ar–CH_3); FAB-MS: $m/z = 296.3$ ($M + H^+$). Anal. Calcd. for $C_{17}H_{17}N_3O_2$:C, 69.14; H, 5.80; N, 14.23. Found C, 69.35; H, 5.86; N, 14.36%.

4.1.52. 6,7-Dimethoxy-2-p-tolylquinazolin-4-amine (**19g**)

Following a procedure identical to that of **19b**, but using compound **18g** (0.1 g, 0.31 mmol) as starting material, compound **19g** was obtained as a white solid (0.07 g, 76%): ^{1}H NMR: 8.35–8.33 (d, 2H, Ar–*H*; $J = 8.2$ Hz), 7.31 (s, 1H, Ar–*H*), 7.29–7.27 (d, 2H, Ar–*H*; $J = 8.0$ Hz), 6.92 (s, 1H, Ar–*H*), 5.45 (b, 2H, N–H_2), 4.04 (s, 3H, O–CH_3), 3.99 (s, 3H, O–CH_3) and 2.42 (s, 3H, CH_3); FAB-MS: $m/z = 296.4$ ($M + H^+$). Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found C, 69.27; H, 5.76; N, 14.46%.

4.1.53. 6,7-Dimethoxy-N^2-(4-nitrophenyl)quinazoline-2,4-diamine (**22a**)

A mixture of 4-nitroaniline (**20a**) (0.43 g, 3.12 mmol) and 4-amino-2-chloro-6,7-dimethoxyquoinazoline (**21**) (0.2 g, 0.84 mmol) in DMF (3 mL) was stirred at 100 °C for 16 h. The reaction mixture was cooled to rt and quenched in diethyl ether (30 mL). The solid thus obtained was filtered, washed with diethyl ether (2 × 10 mL) followed by methanol (2 × 10 mL) and dried. The solid residue was recrystallized from a mixture of chloroform and methanol to afford compound **22a** (0.15 g, 43%): ^{1}H NMR: 8.09–8.00 (m, 5H, Ar–*H*), 6.90 (s, 1H, Ar–*H*) 4.15 (bs, 3H, N–H_2 and N–*H*) and 3.94–3.90 (m, 6H, 2× O–CH_3); FAB-MS: $m/z = 342.3$ ($M + H^+$). Anal. Calcd. for $C_{16}H_{15}N_5O_4$:C, 56.30; H, 4.43; N, 20.52. Found C, 56.02; H, 4.63; N, 20.14%.

4.1.54. N-{4-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)amino] biphenyl-2-yl}methanesulfonamide (**22b**)

Following a procedure identical to the one as described for **22a**, but using 4-amino-2′-methanesulfonamidobiphenyl (**20b**) (1.63 g, 6.25 mmol), compound **22b** was obtained as solid (0.81 g, 83%): ^{1}H NMR: 13.27 (b, 1H, N–*H*), 10.46 (s, 1H, N–*H*), 7.88–7.86 (d, 2H, Ar–*H*; $J = 8.6$ Hz), 7.81 (b, 1H, N–*H*), 7.69 (s, 1H, Ar–*H*), 7.54–7.52 (m, 1H, Ar–*H*), 7.43–7.41 (d, 2H, Ar–*H*; $J = 8.6$ Hz), 7.39–7.34 (m, 1H, Ar–*H*), 7.30–7.27 (m, 2H, Ar–*H*), 6.96 (s, 1H, Ar–*H*), 4.01 (s, 3H, O–CH_3), 3.97 (s, 3H, O–CH_3) and 2.84 (s, 3H, CH_3); FAB-MS: $m/z = 466.5$ ($M + H^+$). Anal. Calcd. for $C_{23}H_{23}N_5O_4S$: C, 59.35; H, 4.98; N, 15.04. Found C, 59.03; H, 4.78; N, 15.43%.

4.2. Cytotoxicity

4.2.1. Cell culture

Colon cancer cells, HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$were kindly provided by Dr. Bert Vogelstein (Johns Hopkins Medical Institutions, Baltimore, MD). The human ovarian carcinoma cell line (HEY) naturally resistant to cisplatin (CDDP) was kindly provided by Dr. Louis Dubeau (USC Norris Cancer Center). Cells were maintained as monolayer cultures in RPMI 1640. Media were supplemented with 10% fetal bovine serum (Gemini-Bioproducts, Woodland, CA) and 2 mmol/L L-Glutamine at 37 °C in a humidified atmosphere of 5% CO_2. To remove the adherent cells from the flask for passaging and counting, cells were washed with PBS without calcium or magnesium, incubated with a small volume of 0.25% trypsin–EDTA solution (Sigma–Aldrich, St. Louis, MO) for 5–10 min, and washed with culture medium and centrifuged. All experiments were performed using cells in exponential growth. Cells were routinely checked for *Mycoplasma* contamination using a PCR-based assay (Stratagene, Cambridge, UK).

4.2.2. Drugs

A 10 mM stock solution of all compounds were prepared in DMSO and stored at −20 °C. Further dilutions were freshly made in PBS or cell-culture media.

4.2.3. Cytotoxicity assays

Cytotoxicity was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [13]. Briefly, cells were seeded in 96-well microtiter plates and allowed to attach. Cells were subsequently treated with a continuous exposure to the corresponding drug for 72 h. A MTT solution (at a final concentration of 0.5 mg/mL) was added to each well and cells were incubated for 4 h at 37 °C. After removal of the medium, DMSO was added and the absorbance was read at 570 nm. All assays were done in triplicate. Percentage of cell growth inhibition was expressed as: $(1 - A/C) \times 100\%$ (A and C were the absorbance values from experimental and control cells, respectively). Inhibitory concentration 50% (IC_{50}) values were determined for each drug from a plot of log (drug concentration) versus percentage of cell growth inhibition. Standard deviation was calculated based on the IC_{50}values obtained from at least three independent experiments.

4.2.4. Colony formation assay

Colony formation assays were also performed to confirm the activity of these compounds as described [14]. Briefly, cells were plated in 6-well plates at a density of 100 cells/well and allowed to attach. Next day, serial dilutions of the corresponding compounds were added for 24 h. After exposure, cells were washed in PBS and cultured in free media until colonies were formed (8–10 days). Cells were subsequently washed, fixed with a glutaraldehyde 1% solution for 30 min and stained with a solution of crystal violet (2%) for 30 min. After staining, cells were thoroughly washed with water. Colonies were imaged on the VersaDoc Imaging System (BioRad) and counted using the Quantity One software package (BioRad). The data reported represent the mean of a minimum of three independent experiments.

4.2.5. Cell cycle analysis

Cell cycle perturbations were analyzed by propidium iodide DNA staining. Briefly, exponentially growing cells were treated with different doses of the compounds for various times. At the end of each treatment time, cells were collected and washed with PBS after a gentle centrifugation at 1000 rpm for 5 min. Cells were thoroughly resuspended in 0.5 mL of PBS and fixed in 70% ethanol for at least 2 h at 4 °C. Ethanol-resuspended cells were then centrifuged at 1000 rpm for 5 min and washed twice in PBS to remove residual ethanol. For cell cycle analysis, the pellets were resuspended in 1 mL of PBS containing 0.02 mg/mL of propidium iodide, 0.5 mg/mL of DNase-free RNase A and incubated at 37 °C for 30 min. Cell cycle profiles were obtained using BD LSRII flow cytometer (BD Bioscience, San Jose, CA, USA) and data were analyzed with BD FACSDiva software package (BD Biosciences, CA, USA).

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